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APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: IMIDAZOLAMINO COMPOUNDS

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Imidazolamino Compounds

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 60/406,363, filed August 26, 2002, the contents of which are incorporated herein by reference.

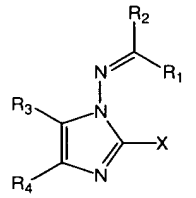
BACKGROUND

Cancer is one of the most common causes of death in developed countries. Despite continuing advances, most existing cancer treatments have undesirable side effects and limited efficacy. Identifying new effective anti-cancer drugs has always been the focus of cancer research.

Imidazolamino compounds have been demonstrated in animal models of non-insulin-dependent diabetes mellitus to both improve insulin sensitivity and promote weight loss selectively from adipose tissue. Indeed, some of them are anti-diabetic drugs. See, e.g., J. Med. Chem., 2001, 44, 1231-1248. However, no imidazolamino compounds have been reported to possess anti-cancer activities.

SUMMARY

One aspect of this invention relates to imidazolamino compounds of formula (I)



(I)

In formula (I), X is $-NR_aR_b$ or $-N=CR_cR_d$, in which each of R_a and R_b, independently, is hydrogen, halo, alkyl, haloalkyl, arylalkyl, heteroarylalkyl, arylcarbonyl, heteroarylcarbonyl, arylaminocarbonyl, or arylsulfonyl, in which aryl or heteroaryl is optionally substituted with alkoxy, halo, nitro, cyano, haloalkyl; and each of R_c and R_d, independently, is hydrogen; halo; alkyl; heteroaryl; phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, alkoxy, or amino; phenylsulfonyl substituted with cyano, halo, oxo, or amino; phenylcarbonyl substituted with cyano, halo, oxo, or amino; naphthylsulfonyl substituted with cyano, halo, oxo, or amino; naphthylcarbonyl substituted with cyano, halo, oxo, or amino; or alkyl optionally substituted

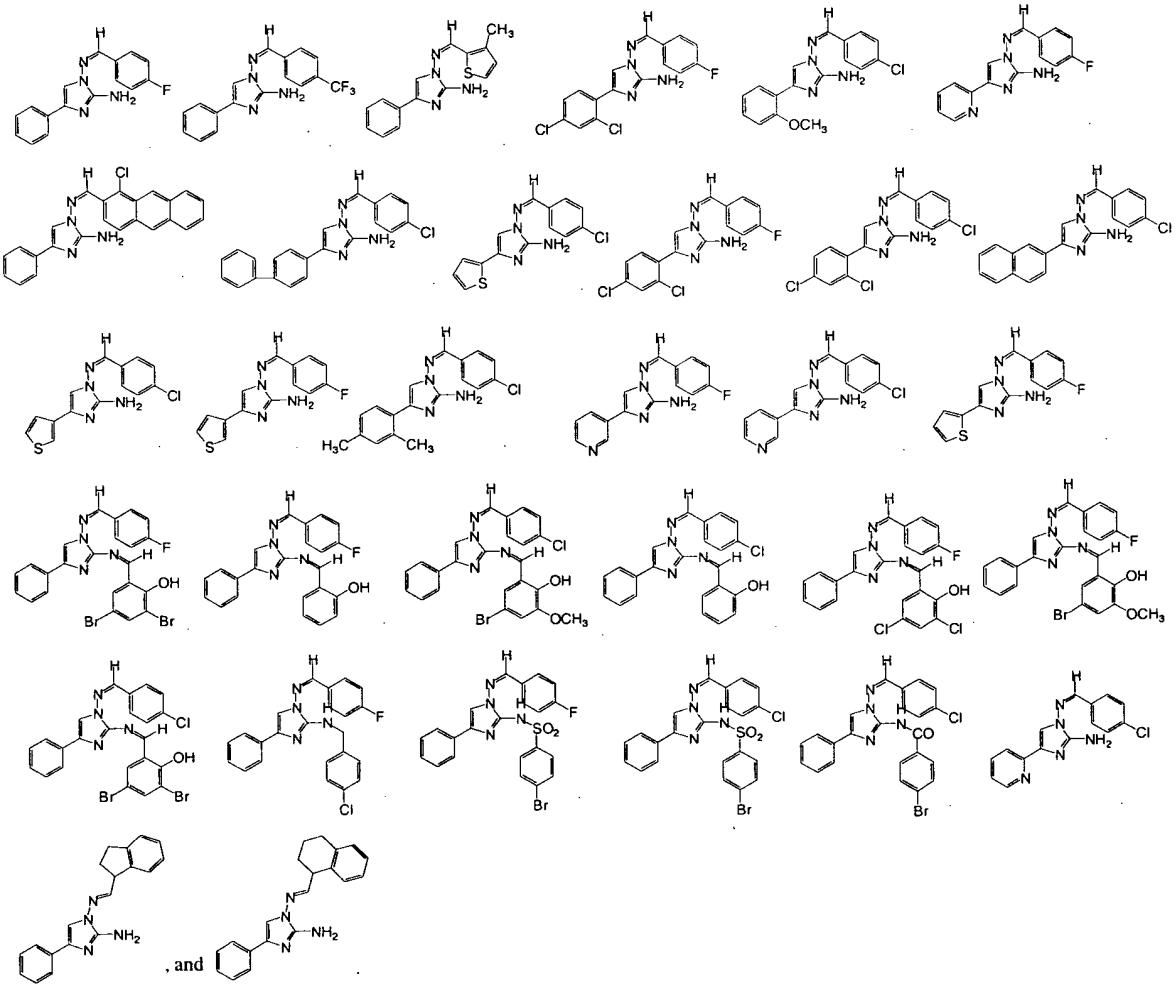
with halo, phenyl or imidazolyl, or phenyl or imidazolyl optionally substituted with alkyl, halo, or hydroxy. R_1 is cycloalkyl, cycloalkenyl, aryl, heteroaryl, or heterocyclyl, optionally fused to aryl, heteroaryl, cycloalkyl, or heterocyclyl; hydrogen; halo; alkyl; haloalkyl; alkenyl; or alkynyl. R_2 is hydrogen, alkyl, cycloalkyl, cycloalkenyl, phenyl, thienyl, thiazolyl, anthryl, or quinolyl, optionally substituted with hydroxy, halo, cyano, alkyl, haloalkyl, nitro, or alkoxy. R_3 is hydrogen, alkyl, or phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, cyano, nitro, or alkoxy. R_4 is diphenyl, thienyl, pyridinyl, thiazolyl, anthryl, naphthyl, or quinolyl, optionally substituted with hydroxy, halo, alkyl, haloalkyl, nitro, or alkoxy, when R_2 is thienyl, thiazolyl, anthryl, or quinolyl, optionally substituted with hydroxy, halo, alkyl, haloalkyl, cyano, nitro, or alkoxy; is diphenyl, thienyl, pyridinyl, thiazolyl, anthryl, naphthyl, or quinolyl, optionally substituted with hydroxy, halo, alkyl, haloalkyl, cyano, nitro, or alkoxy, when R_2 is phenyl optionally substituted with hydroxy, alkyl, haloalkyl, or alkoxy; is pyridinyl, thiazolyl, anthryl, naphthyl, or quinolyl, optionally substituted with hydroxy, halo, alkyl, haloalkyl, nitro, or alkoxy, when R_2 is phenyl optionally substituted with chloro, bromo, iodo, or nitro; is phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, nitro, or alkoxy when R_2 is phenyl substituted with fluoro, alkyl, or haloalkyl; or is alkyl, cycloalkyl, cycloalkenyl, or heterocyclyl optionally substituted with hydroxy, halo, alkyl, cyano, nitro, haloalkyl or alkoxy, when R_2 is hydrogen, alkyl, cycloalkyl, cycloalkenyl, thienyl, thiazolyl, anthryl, or quinolyl, optionally substituted with hydroxy, halo, alkyl, haloalkyl, cyano, or alkoxy.

The term "halo" refers to any radical of fluorine, chlorine, bromine or iodine. The term "alkyl," independently or as a prefix (e.g., alkoxy) or surfix (e.g., haloalkyl), refers to a hydrocarbon chain that may be a straight chain or branched chain of C_{1-12}. The term "alkylene" refers to a divalent alkyl (i.e., -R-) of C_{1-12}. The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like. The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system comprising 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like. The term "heteroarylalkyl" or

the term "heteroaralkyl" refers to an alkyl substituted with a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy substituted with heteroaryl.

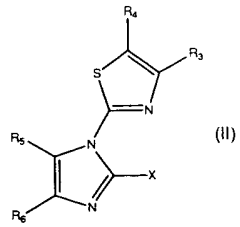
Subsets of the compounds include those in which R_2 is thienyl, thiazolyl, anthryl, or quinonlyl, optionally substituted with hydroxy, halo, alkyl, haloalkyl, nitro, or alkoxy; or phenyl substituted with hydroxy, fluoro, chloro, bromo, alkyl, or alkoxy; those in which R_4 is phenyl, pyridinyl, thiazolyl, anthryl, or quinonlyl, optionally substituted with hydroxy (e.g., hydroxyanthryl), halo (e.g., chloropyridinyl), alkyl (e.g., alkylphenyl), haloalkyl, nitro (e.g., nitrothiazolyl), or alkoxy; and those in which X is NH_2; those in which R_1 is hydrogen or heteroaryl; and R_3 is hydrogen or phenyl.

Specific examples of these compounds include



, and

Another aspect of this invention relates to imidazolamino compounds of formula (II):



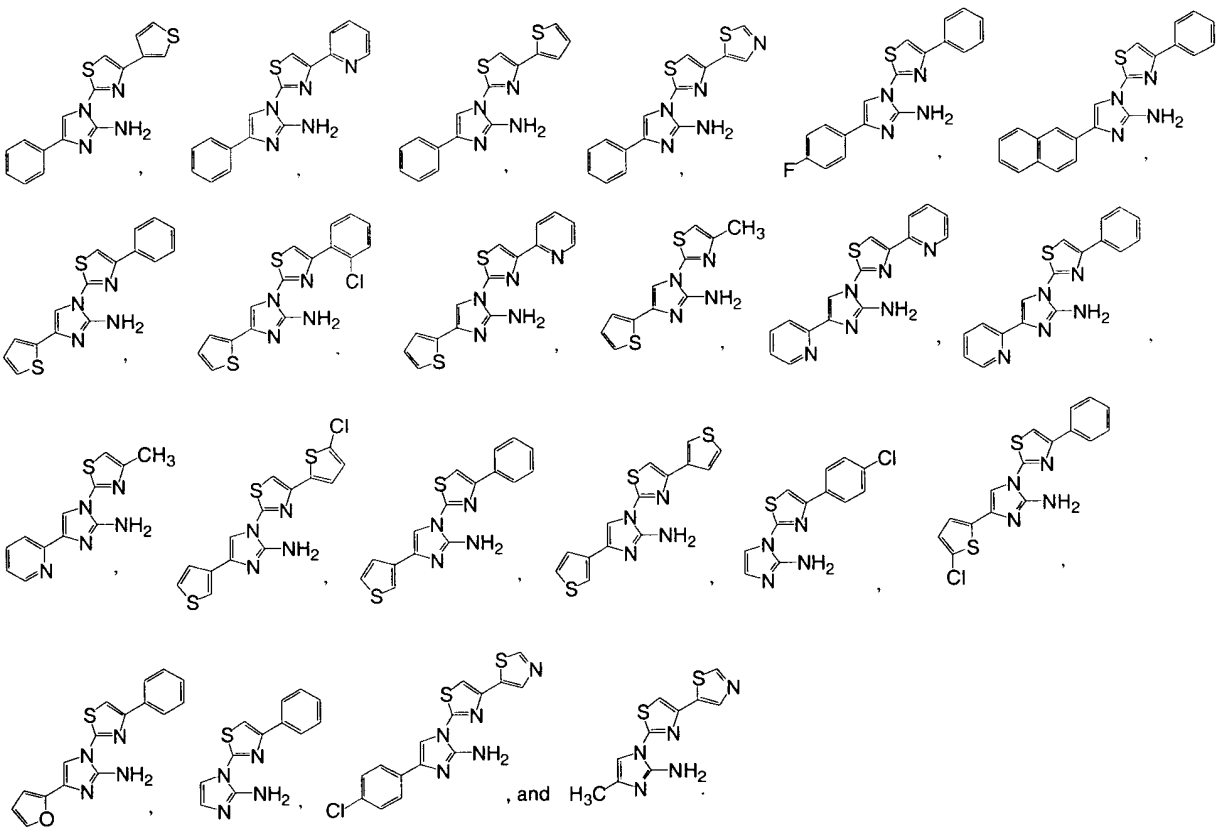
(II)

In formula (II), X is $-NR_aR_b$ or $-N=CR_cR_d$, in which each of R_a and R_b, independently, is hydrogen, halo, alkyl, or haloalkyl; arylalkyl, heteroarylalkyl, arylcarbonyl, heteroarylcarbonyl, arylaminocarbonyl, or arylsulfonyl, in which aryl or heteroaryl is optionally substituted with alkoxy, halo, nitro, cyano, haloalkyl, and each of R_c and R_d, independently, is hydrogen; halo; alkyl; heteroaryl; phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, alkoxy, or amino; phenylsulfonyl substituted with cyano, halo, oxo, or amino; phenylcarbonyl substituted with cyano, halo, oxo, or amino; naphthylsulfonyl substituted with cyano, halo, oxo, or amino; naphthylcarbonyl substituted with cyano, halo, oxo, or amino; or alkyl optionally substituted with halo, phenyl or imidazolyl, or phenyl or imidazolyl optionally substituted with alkyl, halo, or hydroxy. Each of R_1 and R_2, independently, is hydrogen, alkyl, or haloalkyl. R_3 is alkyl, phenyl, thienyl, pyridinyl, thiazolyl, cycloalkyl, cycloalkenyl, benzofuranyl, indolyl, pyrazinyl, pyrimidinyl, pyrrolyl, N-methylpyrrolyl, isothiazolyl, oxadiazolyl, furyl, isoazolyl, oxazolyl, or heterocyclyl optionally substituted with halo, alkyl, haloalkyl, hydroxy, or amino. R_4 is hydrogen, alkyl, hydroxy, or amino. R_5 is hydrogen, alkyl, or aryl optionally substituted with hydroxy, halo, alkyl, haloalkyl, or amino. R_6 is hydrogen, fluorophenyl, naphthyl, thienyl, pyridinyl, furyl, thiazolyl cycloalkyl, cycloalkenyl, benzofuranyl, indolyl, pyrazinyl, pyrimidinyl, pyrrolyl, N-methylpyrrolyl, isothiazolyl, oxadiazolyl, isoazolyl, oxazolyl, or heterocyclyl when R_3 is alkyl optionally substituted with halo, hydroxy, or amino, or is phenyl optionally substituted with halo, hydroxy, amino, or alkyl; and R_5 is hydrogen, alkyl, or aryl optionally substituted with hydroxy, alkyl, or amino, or is phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, or amino when R_3 is thienyl, pyridinyl, or thiazolyl, optionally substituted with halo, alkyl, haloalkyl, or hydroxy, and R_5 is hydrogen, alkyl, or phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, or amino

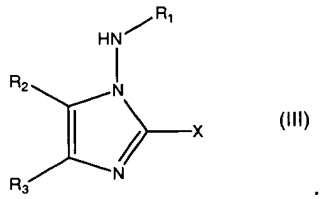
Subset of the compounds of formula (II) include those in which R_6 is hydrogen, fluorophenyl, naphthyl, thienyl, pyridinyl, furyl, or thizaolyl, when R_3 is alkyl optionally substituted with halo, hydroxy, or amino, or is phenyl optionally substituted with halo, hydroxy,

amino, or alkyl; and when R_5 is hydrogen, alkyl, or aryl optionally substituted with hydroxy, alkyl, or amino; those in which X is NH_2; those in which R_4 is H; those in which R_3 is phenyl or alkyl, optionally substituted with halo; and R_5 is hydrogen or phenyl.

Specific examples of the compound of formula (II) include



Still another aspect of this invention relates to imidazolamino compounds of formula (III):



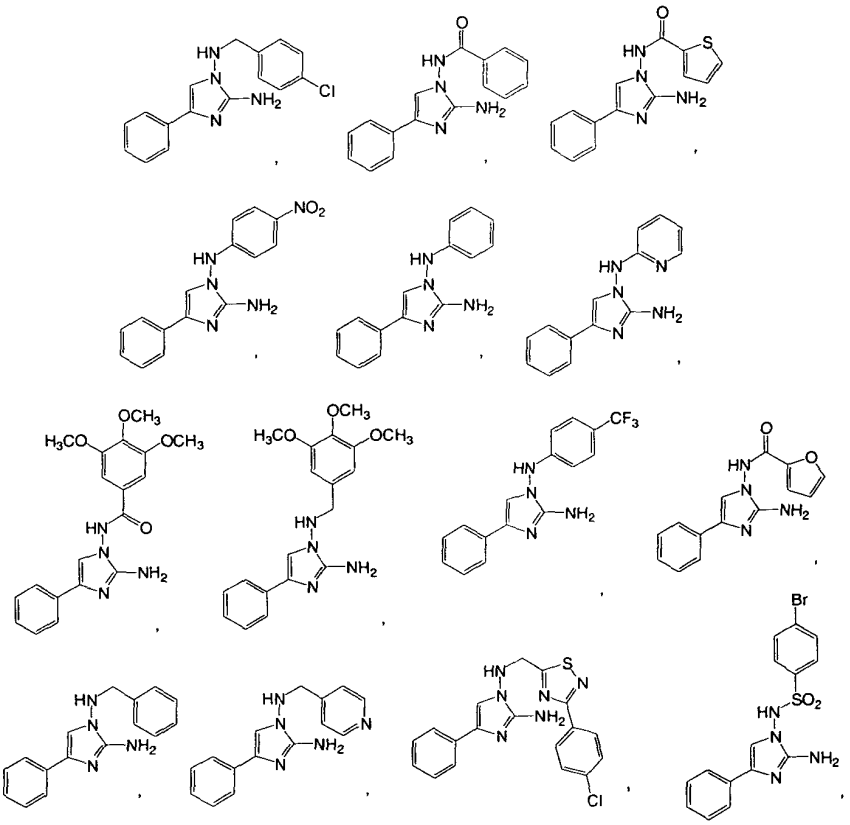
(III)

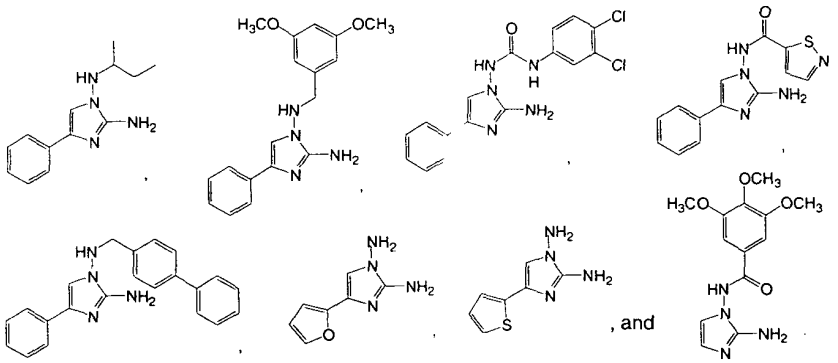
In formula (III), X is $-NR_aR_b$ or $-N=CR_cR_d$, in which each of R_a and R_b, independently, is halo or haloalkyl, arylalkyl, heteroarylalkyl, arylcarbonyl, heteroarylcarbonyl, arylaminocarbonyl, or arylsulfonyl, in which aryl or heteroaryl is optionally substituted with alkoxy, halo, nitro, cyano, haloalkyl, or phenyl optionally substituted with halo; and each of R_c and R_d, independently, is hydrogen, halo, or phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, alkoxy, or amino; phenylsulfonyl substituted with cyano, halo, oxo, or amino; phenylcarbonyl substituted

with cyano, halo, oxo, or amino; naphthalenylsulfonyl substituted with cyano, halo, oxo, or amino; naphthylcarbonyl substituted with cyano, halo, oxo, or amino; or alkyl optionally substituted with halo, phenyl optionally substituted with alkyl, halo, or hydroxy, or imidazolyl optionally substituted with alkyl, halo, or hydroxy; R_1 is alkyl, phenyl, haloalkylphenyl, phenylalkyl, diphenylalkyl, pyridinylalkyl, phenyloxadiazolylalkyl, phenylcarbonyl, furylcarbonyl, thienylcarbonyl, isoxazolylcarbonyl, phenylaminocarbonyl, or phenylsulfonyl, optionally substituted with alkoxy, halo, cyano, nitro, or haloalkyl; or hydrogen; R_2 is hydrogen, alkyl, phenyl, furyl, thienyl, pyridinyl, oxadiazolyl, or isoxazolyl, optionally substituted with halo; and R_3 is hydrogen, furyl, thienyl, pyridinyl, oxadiazolyl, or isoxazolyl, optionally substituted with halo or alkyl. Alternatively, X is $-NR_aR_b$ or $-N=CR_cR_d$, in which each of R_a and R_b, independently, is halo or haloalkyl, arylalkyl, heteroarylalkyl, arylcarbonyl, heteroarylcarbonyl, arylaminocarbonyl, or arylsulfonyl, in which aryl or heteroaryl is optionally substituted with alkoxy, halo, nitro, cyano, haloalkyl, or phenyl optionally substituted with halo; and each of R_c and R_d, independently, is hydrogen, halo, or phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, alkoxy, or amino; phenylsulfonyl substituted with cyano, halo, oxo, or amino; phenylcarbonyl substituted with cyano, halo, oxo, or amino; naphthalenylsulfonyl substituted with cyano, halo, oxo, or amino; naphthylcarbonyl substituted with cyano, halo, oxo, or amino; or alkyl optionally substituted with halo, phenyl optionally substituted with alkyl, halo, or hydroxy, or imidazolyl optionally substituted with alkyl, halo, or hydroxy; R_1 is alkyl, haloalkylphenyl, phenylalkyl, diphenylalkyl, pyridinylalkyl, phenyloxadiazolylalkyl, phenylcarbonyl, furylcarbonyl, thienylcarbonyl, isoxazolylcarbonyl, phenylaminocarbonyl, or phenylsulfonyl, optionally substituted with alkoxy, halo, nitro, or haloalkyl; or hydrogen; R_2 is hydrogen, alkyl, phenyl, furyl, thienyl, pyridinyl, oxadiazolyl, or isoxazolyl, optionally substituted with halo; and R_3 is phenyl optionally substituted with halo, alkoxy or alkyl. As yet another alternative, X is $-NR_aR_b$ or $-N=CR_cR_d$, in which each of R_a and R_b, independently, is hydrogen or alkyl, arylalkyl, heteroarylalkyl, arylcarbonyl, heteroarylcarbonyl, arylaminocarbonyl, or arylsulfonyl, in which aryl or heteroaryl is optionally substituted with alkoxy, halo, nitro, cyano, haloalkyl, or phenyl optionally substituted with halo; and each of R_c and R_d, independently, is hydrogen, halo, or phenyl optionally substituted with hydroxy, halo, alkyl, haloalkyl, alkoxy, or amino; phenylsulfonyl substituted with cyano, halo, oxo, or amino; phenylcarbonyl substituted with cyano, halo, oxo, or amino; naphthalenylsulfonyl substituted

with cyano, halo, oxo, or amino; naphthylcarbonyl substituted with cyano, halo, oxo, or amino; or alkyl optionally substituted with halo, phenyl optionally substituted with alkyl, halo, or hydroxy, or imidazolyl optionally substituted with alkyl, halo, or hydroxy; R_1 is hydrogen, alkyl, phenyl, haloalkylphenyl, phenylalkyl, diphenylalkyl, pyridinylalkyl, phenyloxadiazolylalkyl, phenylcarbonyl, furylcarbonyl, thienylcarbonyl, isoxazolylcarbonyl, phenylaminocarbonyl, or phenylsulfonyl, in which phenyl, furyl, thienyl, pyridinyl, oxadiazolyl, or isoxazolyl is optionally substituted with alkoxy, halo, nitro, or haloalkyl; R_2 is hydrogen, alkyl, phenyl, furyl, thienyl, pyridinyl, oxadiazolyl, or isoxazolyl, optionally substituted with halo; and R_3 is hydrogen, furyl, thienyl, pyridinyl, oxadiazolyl, or isoxazolyl, optionally substituted with halo or alkyl.

Subsets of the compounds of formula (III) includes those in which X is NH_2; those in which R_2 is hydrogen; and those in which R_3 is phenyl, furyl, or thienyl. Specific examples of these compounds include





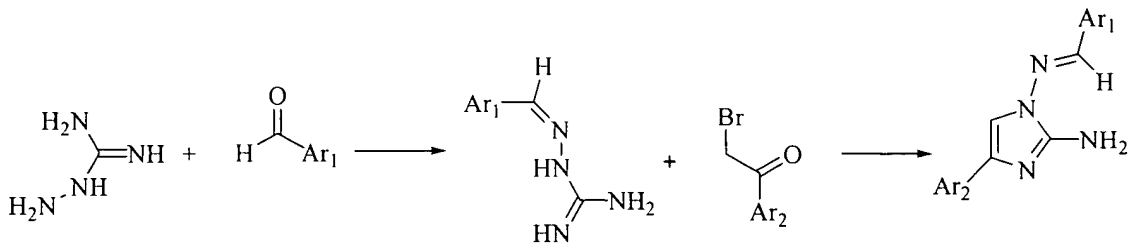
The imidazolamino compounds described above include their salts, if applicable. Such a salt, for example, can be formed between a positively charged substituent, e.g., amino, and an anion. Suitable anions include, but are not limited to, chloride, bromide, iodide, sulfate, nitrate, phosphate, or acetate. Likewise, a negatively charged substituent (e.g., carboxylate) can form a salt with a cation. Suitable cations include, but are not limited to, sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion.

The compounds of this invention can be used as anti-cancer drugs. Thus, also within the scope of this invention are pharmaceutical compositions each containing a pharmaceutically acceptable carrier and one of the above-described compounds; and methods for treating cancer, which include administering to a subject in need thereof an effective amount of an above-described compound.

The details of several embodiments of the invention are set forth in the accompanying description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DETAILED DESCRIPTION

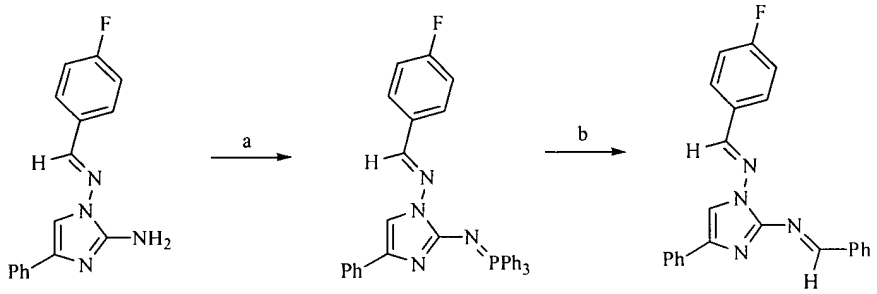
Imidazolamino compounds of this invention generally can be synthesized from an aminoguanidine compound by first coupling it with a carbonyl containing compound (e.g., an aldehyde) to form an iminoguinidine adduct. The iminoguinidine adduct can then react with a haloaceto compound (e.g., bromoacetofuryl) via a ring-form mechanism to form an imidazolyl ring. Shown below is Scheme 1, which depicts a method for preparing some imidazolamino compounds of this invention.



Scheme 1

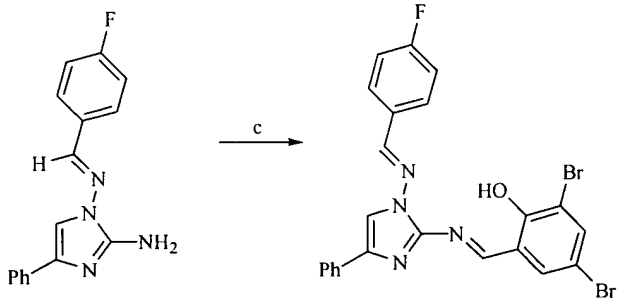
Starting materials containing different substituents on Ar_1 and Ar_2 can be used to prepare imidazolamino compounds containing various substituents on Ar_1 or Ar_2 by following the same reaction scheme.

The 2-amino group in the imidazolamino compounds can be modified as shown below in Schemes 2, 3, and 4:



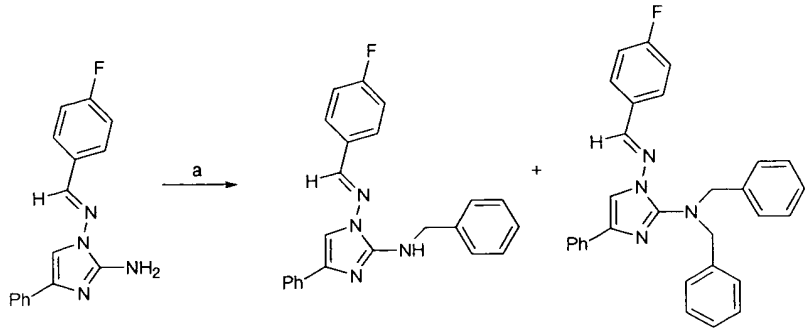
(a) Ph_3PBr_2, Et_3N, benzene; (b) benzaldehyde, toluene, 3 h.

Scheme 2



(c) 3, 5-dibromosalicylaldehyde, EtOH, 70°C.

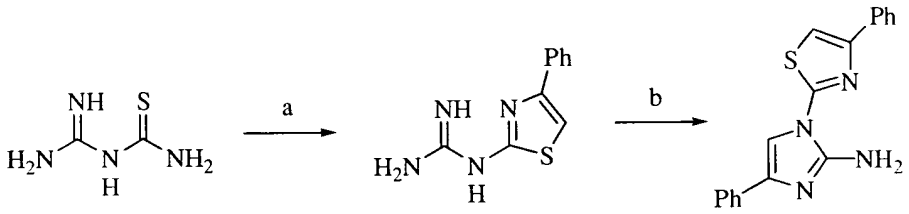
Scheme 3



(a) benzyl bromide, THF, 80 °C.

Scheme 4

The imidazolamino compounds containing a thiazolyl ring at 1-N can be synthesized, as shown below in Scheme 5, by using amidinothiourea in a method similar to that depicted in Scheme 1:



(a) 2-bromoacetophenone, EtOH, 80°C; (b) 2-bromoacetophenone, EtOH, 80°C.

Scheme 5

Like other imidazolamino compounds, the 2-amino group of the above-shown thiazolylimidazolamino compound can be modified as shown in Schemes 2-4.

As mentioned above, the compounds of this invention can be used to inhibit the growth of (including killing) cancer cells. Thus, another aspect of this invention relates to a pharmaceutical composition which contains an effective amount of at least one of the compounds described above (or its salt) and a pharmaceutically acceptable carrier for treating cancer. "An effective amount" refers to the amount of the compound which is required to confer a therapeutic effect on the treated subject. The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described in Freireich et al., Cancer Chemother. Rep., **1966**, 50, 219. Body surface area may be approximately determined from height and weight of the patient. See, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardley, N.Y., **1970**,

537. Effective doses will also vary, as recognized by those skilled in the art, depending on route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatments including use of other anti-platelet aggregation agents. Examples of the carriers include colloidal silicon dioxide, magnesium stearate, cellulose, sodium lauryl sulfate, and D&C Yellow # 10.

The pharmaceutical composition may be administered via a parenteral route, e.g., topically, subcutaneously, intraperitoneally, intramuscularly, and intravenously. Examples of parenteral dosage forms include aqueous solutions of the active compound, in an isotonic saline, 5% glucose, or any other well-known pharmaceutically acceptable carrier. Solubilizing agents, such as cyclodextrins, or other solubilizing agents well known to those familiar with the art, can also be included in the pharmaceutical composition.

An imidazolamino compound of this invention can be formulated into dosage forms for other routes of administration (e.g., orally, mucosally, or percutaneously) utilizing well known methods. The pharmaceutical composition can be formulated, for example, in dosage forms for oral administration in a capsule, a gel seal, or a tablet. Capsules may comprise any well known pharmaceutically acceptable material such as gelatin or cellulose derivatives. Tablets may be formulated in accordance with the conventional procedure by compressing mixtures of the active compounds, a solid carrier, and a lubricant. Examples of solid carriers include starch and sugar bentonite. The compound can also be administered in a form of a hard shell tablet or capsule containing, for example, lactose or mannitol as a binder, a conventional filler, and a tableting agent.

As mentioned above, the imidazolamino compounds of this invention have anti-cancer activities. Their activities can be evaluated by *in vitro* and *in vivo* assays well known in the art. For instance, a panel of human cancer cell lines can be first seeded in a medium, incubated, and brought in contact with a compound. The anti-cancer (cytotoxic) acitivities can then be determined by evaluating the viability of the cells. An *in vivo* assay can be conducted by using mice inoculated with cancer cells, followed by administration of the compounds of this invention. The efficacy of the compound can then be confirmed by monitoring the survival rate of these mice.

Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications recited

herein are hereby incorporated by reference in their entirety. The following specific examples, which describe synthesis and biological testing of various compounds of the present invention, are therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Exampe 1:

Synthesis of *N*1-[(*E*)-1-(4-fluorophenyl)methylidene]-4-phenyl-1*H*-1,2-imidazolediamine (Compound 1)

A mixture of 4-fluorobenzaldehyde (2.0 g, 15.8 mmol), 20% hydrochloric acid (4 mL) was added to an aminoguanidine bicarbonate (2.2 g, 15.8 mmol) aqueous solution. After completion of the liberation of carbon dioxide, the mixture was heated for 4 h. A solution of 40 % aqueous potassium hydroxide (7 mL) was added and the mixture was heated at reflux for additional 10 min. The resulting solution was filtered, washed with water until the wash water was at pH 7, dried and recrystallized from ethanol to give 2-[(*E*)-1-(4-fluorophenyl)methylidene]-1-hydrazinecarboximidamide (2.6 g, 91%) as a yellow solid.

2-[(*E*)-1-(4-fluorophenyl)methylidene]-1-hydrazinecarboximidamide (0.74 g, 4.06 mmol) was added to a solution of 2-bromoacetophenone (0.40 g, 2.03 mmol) in ethanol (10 mL), and the reaction was heated to 70°C for 4 h. A solution of sodium hydroxide aqueous solution was added dropwise. The yellow precipitate was formed and cooled the reaction mixture at room temperature for additional 10 h, then filtered, washed with hot water and recrystallized from ethanol to give **1** (0.44 g, 77%) as a yellow solid.

^{1}H NMR (DMSO-d_6): δ 8.56 (s, 1H), 8.01-7.96 (m, 3H), 7.69 (d, J = 7.2 Hz, 2H), 7.37-7.30 (m, 4H), 7.18 (t, J = 7.2 Hz, 1H), 6.20 (s, 2H). ESMS *m/z*: 281.5 (MH^+).

Example 2:

Synthesis of *N*1-[(*E*)-1-(4-chlorophenyl)methylidene]-4-(5-chloro-2-thienyl)-1*H*-1,2-imidazolediamine (Compound 2)

A mixture of 4-chlorobenzaldehyde (2.0 g, 10.2 mmol), 20 % hydrochloric acid (4 mL) was added to an aminoguanidine bicarbonate (1.39 g, 10.2 mmol) aqueous solution, and heated to reflux for 4 h. A solution of 40 % aqueous potassium hydroxide (7 mL) was added dropwise, the mixture was heated at reflux for 10 min and the precipitate formed. The precipitate was

filtered, washed with water, and recrystallized from ethanol to give 2-[(*E*)-1-(4-chlorophenyl)methylidene]-1-hydrazinecarboximidamide (1.77 g, 88 %) as a yellow solid.

A solution of 2-bromo-1-(5-chloro-2-thienyl)-1-ethanone (1.07 g, 4.49 mmol), 2-[(*E*)-1-(4-chlorophenyl)methylidene]-1-hydrazinecarboximidamide (1.77 g, 8.98 mmol) was heated in ethanol (10 mL) for 2 h, then the mixture was left at room temperature for additional 10 h. The precipitate filtered, washed with hot water, and recrystallized from ethanol to give *N*1-[(*E*)-1-(4-chlorophenyl)methylidene]-4-(5-chloro-2-thienyl)-1*H*-1,2-imidazolediamine **2** (1.09 g, 72%) as a brown solid.

^{1}H NMR (DMSO-d_6): δ 8.51 (s, 1H), 7.94 (d, J = 7.8 Hz, 2H), 7.86 (s, 1H), 7.57 (d, J = 7.8 Hz, 2H), 7.05 (s, 2H), 6.39 (s, 2H). ESMS *m/z*: 337.4 (MH^+).

Example 3:

Synthesis of *N*1-[(*E*)-1-(4-methoxyphenyl)methylidene]-4,5-diphenyl-1*H*-1,2-imidazolediamine (Compound 3)

A mixture of 4-anisaldehyde (2.0 g, 14.4 mmol), 20% hydrochloric acid (4 mL) was added to an aminoguanidine bicarbonate (1.96 g, 14.4 mmol) aqueous solution, and heated to reflux for 4 h. 40% aqueous potassium hydroxide (7 mL) was added and the mixture was heated at reflux for additional 10 min. The mixture was cooled in room temperature for 10 h and a yellow precipitate formed. The yellow precipitate was filtered, washed with hot water and recrystallized from ethanol to give 2-[(*E*)-1-(4-methoxyphenyl)methylidene]- 1-hydrazinecarboximidamide (2.4 g, 87%) as a yellow solid.

A solution of desyl bromide (1.72 g, 6.26 mmol), and 2-[(*E*)-1-(4-methoxyphenyl) methylidene]-1-hydrazinecarboximidamide (2.4 g, 12.5 mmol) was heated at reflux in ethanol (10 mL) for 2 h. The mixture was cooled at room temperature for additional 10 h, and a yellow precipitate formed. The yellow precipitate was filtered, washed with hot water and recrystallized from ethanol to give *N*1-[(*E*)-1-(4-methoxyphenyl)methylidene]-4,5-diphenyl -1*H*-1,2-imidazolediamine **3** (1.57 g, 68%) as a yellow solid.

^{1}H NMR (DMSO-d_6): δ 7.91 (s, 1H), 7.52 (d, J = 9.0 Hz, 2H), 7.44-7.31 (m, 6H), 7.19-7.06 (m, 4H), 6.98 (d, J = 9.0 Hz, 2H), 5.92 (br, 2H), 3.79 (s, 3H). ESMS *m/z*: 369.1 (MH^+).

Example 4:

Synthesis of *N*1-[(*E*)-1-(4-fluorophenyl)methylidene]-4-(1,3-thiazol-2-yl)-1*H*-1,2-imidazolediamine (Compound 4)

A mixture of 4-fluorobenzaldehyde (2.0 g, 15.8 mmol), 20% hydrochloric acid (4 mL) was added to an aminoguanidine bicarbonate (2.2 g, 15.8 mmol) aqueous solution. After completion of the liberation of carbon dioxide, the mixture was heated for 4 h. A solution of 40% aqueous potassium hydroxide (7 mL) was added and the mixture was heated at reflux for additional 10 min. The resulting solution was cooled, filtered, washed with water until the wash water was at pH 7, dried and recrystallized from ethanol to give 2-[(*E*)-1-(4-fluorophenyl)methylidene]-1-hydrazinecarboximidamide (2.6 g, 91 %) as a yellow solid.

A solution of 2-bromo-1-(1,3-thiazol-2-yl)-1-ethanone (1.5 g, 7.2 mmol), and 2-[(*E*)-1-(4-fluorophenyl)methylidene]-1-hydrazinecarboximidamide (2.6 g, 14.4 mmol) was heated at reflux in ethanol (10 mL) for 2 h. The mixture was left at room temperature for additional 10 h, then the precipitate was filtered, washed with hot water and recrystallized from ethanol to give *N*1-[(*E*)-1-(4-fluorophenyl)methylidene]-4-(1,3-thiazol-2-yl)-1*H*-1,2-imidazole diamine **4** (1.45 g, 70%) as a yellow solid.

^{1}H NMR (DMSO-d_6): δ 8.74 (s, 1H), 8.12 (s, 1H), 8.03-7.97 (m, 2H), 7.81-7.79 (m, 1H), 7.59-7.58 (m, 1H), 7.35 (t, J = 8.0 Hz, 2H), 6.57 (br, 2H). ESMS *m/z*: 288.0 (MH^+).

Example 5:

Synthesis of 1-(4-methyl-1,3-thiazol-2-yl)-4-phenyl-1*H*-2-imidazolamine (Compound 5)

A mixture of amidinothiourea (2.0 g, 16.9 mmol), chloroacetone (1.56 g, 16.9 mmol) was heated at reflux in acetone (12 mL) for 4 h. The mixture was cooled to room temperature and removed half of the solvent. The precipitate was filtered, washed with dry acetone, and dried to give *N*-(4-methyl-1,3-thiazol-2-yl)guanidine (2.19 g, 83%).

A solution of *N*-(4-methyl-1,3-thiazol-2-yl)guanidine (2.19 g, 14.0 mmol) and 2-bromoacetophenone (13.9 g, 7.0 mmol) in ethanol (5 mL) was heated to 80 °C for 2 h. The mixture was left at room temperature for additional 10 h, filtered, washed with hot water and recrystallized from ethanol to give **5** (1.38 g, 77%).

^{1}H NMR (DMSO-d_6): δ 7.78 (d, J = 7.2 Hz, 2H), 7.63 (s, 1H), 7.35 (t, J = 7.2 Hz, 2H), 7.22 (t, J = 7.2 Hz, 1H), 7.08 (s, 1H), 6.95 (br, 2H), 2.35 (s, 3H). ESMS *m/z*: 257.0 (MH^+), 279.0 $(M+23)^+$.

Example 6:

Synthesis of 1-(4-phenyl-1,3-thiazol-2-yl)-4-(2-thienyl)-1*H*-2-imidazolamine (Compound 6)

A mixture of amidinothiourea (2.0 g, 16.9 mmol), 2-bromoacetophenone (3.4 g, 16.9 mmol) was heated at reflux in acetone (12 mL) for 4 h. The mixture was cooled to room temperature and removed half of the solvent. The precipitate was filtered, washed with acetone, and dried to give *N*-(4-phenyl-1,3-thiazol-2-yl)guanidine (3.2 g, 87%).

A solution of *N*-(4-phenyl-1,3-thiazol-2-yl)guanidine (3.2 g, 14.7 mmol) and 2-bromo-1-(2-thienyl)-1-ethanone (1.5 g, 7.35 mmol) in ethanol (5 mL) was heated to 80 °C for 2 h. The mixture was left at room temperature for additional 10 h, filtered, washed with hot water and recrystallized from ethanol to give **6** (1.48 g, 62%).

^{1}H NMR (DMSO-d_6): δ 7.85 (d, J = 6.9 Hz, 2H), 7.48-7.38 (m, 3H), 7.33 (d, J = 3.3 Hz, 1H), 7.25-7.20 (m, 2H), 7.02-7.03 (m, 2H), 6.36 (br, 2H). ESMS *m/z*: 325.0 (MH^+).

Example 7:

Synthesis of 1-[4-(4-chlorophenyl)-1,3-thiazol-2-yl]-5-phenyl-1*H*-2-imidazolamine (Compound 7)

A mixture of amidinothiourea (2.0 g, 16.9 mmol), 2-bromoacetophenone (3.4 g, 16.9 mmol) was heated at reflux in acetone (12 mL) for 4 h. The mixture was cooled to room temperature and removed half of the solvent. The precipitate was filtered, washed with acetone, and dried to give *N*-(4-phenyl-1,3-thiazol-2-yl)guanidine (3.2 g, 87%).

A solution of *N*-(4-phenyl-1,3-thiazol-2-yl)guanidine (3.2 g, 14.7 mmol) and 2-bromo-2-phenylacetaldehyde (1.46 g, 7.3 mmol) in ethanol (5 mL) was heated to 80°C for 2 h. The mixture was left at room temperature for additional 10 h, filtered, washed with hot water and recrystallized from ethanol to give **7** (1.65 g, 71%).

^{1}H NMR ($CDCl_3$): δ 8.08 (s, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.35-7.19 (m, 5H), 6.82 (s, 1H), 6.50 (br, 2H). ESMS *m/z*: 353.0 (MH^+).

Example 8:

Synthesis of 2,4-dibromo-6-[(1-[(*E*)-1-(4-fluorophenyl)methylidene]amino-4-phenyl-1*H*-2-imidazolyl)imino]methylphenol (Compound 8)

A mixture of 4-fluorobenzaldehyde (2.0 g, 15.8 mmol), 20% hydrochloric acid (4 mL) was added to an aminoguanidine bicarbonate (2.2 g, 15.8 mmol) aqueous solution. After completion of the liberation of carbon dioxide, the mixture was heated for 4 h. A solution of 40 % aqueous potassium hydroxide (7 mL) was added and the mixture was heated at reflux for additional 10 min. The resulting solution was filtered, washed with water until the wash water was at pH = 7, dried and recrystallized from ethanol to give 2-[(*E*)-1-(4-fluorophenyl)methylidene]-1-hydrazinecarboximidamide (2.6 g, 91%) as a yellow solid.

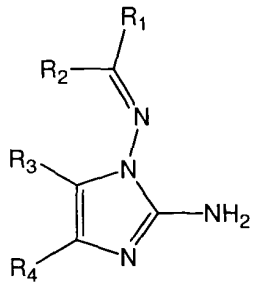
A solution of sodium hydroxide (81 mg, 2.03 mmol) in ethanol (5 mL) was added dropwise over 20 min to a mixture of 2-bromoacetophenone (0.40 g, 2.03 mmol), and 2-[(*E*)-1-(4-fluorophenyl)methylidene]-1-hydrazinecarboximidamide (0.37 g, 2.03 mmol) in ethanol (10 mL). The reaction was heated to 70 °C for 2 h, and a yellow precipitate formed. The mixture was cooled at room temperature for additional 10 h, then filtered, washed with hot water and recrystallized from ethanol to give **1** (0.44 g, 77%) as a yellow solid.

To a solution of *N*1-[(*E*)-1-(4-fluorophenyl)methylidene]-4-phenyl-1*H*-1,2-imidazole diamine **1** (0.44 g, 1.56 mmol) and 3, 5-dibromosalicyclaldehyde (0.44 g, 1.56 mmol) was dissolved in EtOH (20 mL). The mixture was heated to reflux for 2 h, and stirred at room temperature for further 2 h. The resulting solution was filtered, washed with hot water, and recrystallized from ethanol to give **8** (0.7 g, 83%) as a yellow solid.

^{1}H NMR (DMSO-d_6): δ 9.41 (s, 1H), 9.01 (s, 1H), 8.67 (s, 1H), 8.12 (d, J = 2.1 Hz, 1H), 7.98-7.92 (m, 3H), 7.88 (d, J = 8.7 Hz, 2H), 7.49-7.43 (m, 4H), 7.32 (t, J = 7.5 Hz, 1H). ESMS *m/z*: 540.1 (MH^+).

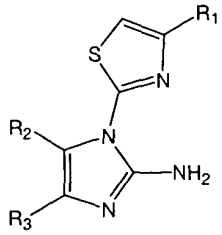
Examples 9-33:

The following compounds were synthesized according to the methods described above:



Compound	R^1	R^2	R^3	R^4
9	3-methyl-2-thienyl	H	H	phenyl
10	4-(trifluoromethyl)phenyl	H	H	phenyl
11	4-chloro-2-nitrophenyl	H	H	phenyl
12	3,4-dichlorophenyl	H	H	phenyl
13	2,3-dichlorophenyl	H	H	Phenyl
14	2-chlorophenyl	H	H	Phenyl
15	4-quinolyl	H	H	Phenyl
16	10-chloro-9-anthryl	H	H	phenyl
17	4-chlorophenyl	H	H	biphenyl
18	4-chlorophenyl	H	H	2-methoxyphenyl
19	4-fluorophenyl	H	H	3-thienyl
20	4-chlorophenyl	H	H	3-thienyl
21	4-chlorophenyl	H	H	2,4-dimethylphenyl
22	4-fluorophenyl	H	H	2-pyridyl
23	4-chlorophenyl	H	H	2-pyridyl
24	4-fluorophenyl	H	H	3-pyridyl
25	4-chlorophenyl	H	H	3-pyridyl
26	4-fluorophenyl	H	H	2-thienyl
27	4-chlorophenyl	H	H	2-thienyl
28	4-fluorophenyl	H	H	2,4-dichlorophenyl
29	4-chlorophenyl	H	H	2,4-dichlorophenyl
30	4-chlorophenyl	H	H	2-naphthyl
31	4-fluorophenyl	H	phenyl	phenyl
32	4-chlorophenyl	H	phenyl	Phenyl
33	4-chlorophenyl	H	H	1,3-thiazol-2-yl

Example 34-59:

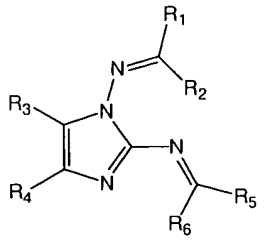


The following compounds were synthesized according to the methods described above:

Compound	R^1	R^2	R^3
34	phenyl	H	phenyl
35	3-thienyl	H	phenyl
36	2-pyridyl	H	phenyl
37	4-chlorophenyl	H	Phenyl
38	5-chloro-2-thienyl	H	phenyl
39	2-thienyl	H	phenyl
40	1,3-thiazol-2-yl	H	phenyl
41	phenyl	H	4-fluorophenyl
42	phenyl	H	2-naphthyl
43	2-chlorophenyl	H	2-thienyl
44	2-pyridyl	H	2-thienyl
45	methyl	H	2-thienyl
46	2-pyridyl	H	2-pyridyl
47	phenyl	H	2-pyridyl
48	methyl	H	2-pyridyl
49	5-chloro-2-thienyl	H	3-thienyl
50	phenyl	H	3-thienyl
51	3-thienyl	H	3-thienyl
52	phenyl	H	5-chloro-2-thienyl
53	3-thienyl	H	3-thienyl
54	phenyl	H	2-furyl
55	phenyl	phenyl	H
56	phenyl	phenyl	phenyl
57	4-chlorophenyl	H	1,3-thiazol-2-yl
58	methyl	H	1,3-thiazol-2-yl
59	phenyl	H	1,3-thiazol-2-yl

Examples 60-65:

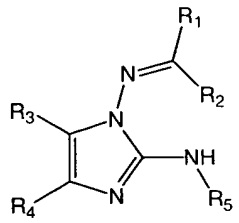
The following compounds were synthesized according to the methods described above:



Compound	R^1	R^2	R^3	R^4	R^5	R^6
60	4-fluorophenyl	H	H	phenyl	2-hydroxyphenyl	H
61	4-chlorophenyl	H	H	phenyl	5-bromo-2-hydroxy-3-methoxyphenyl	H
62	4-chlorophenyl	H	H	phenyl	2-hydroxyphenyl	H
63	4-fluorophenyl	H	H	Phenyl	3,5-dichloro-2-hydroxyphenyl	H
64	4-fluorophenyl	H	H	Phenyl	5-bromo-2-hydroxy-3-methoxyphenyl	H
65	4-chlorophenyl	H	H	1,3-thiazol-2-yl	3,5-dibromo-2-hydroxyphenyl	H

Examples 66-75:

The following compounds were synthesized according to the methods described above:

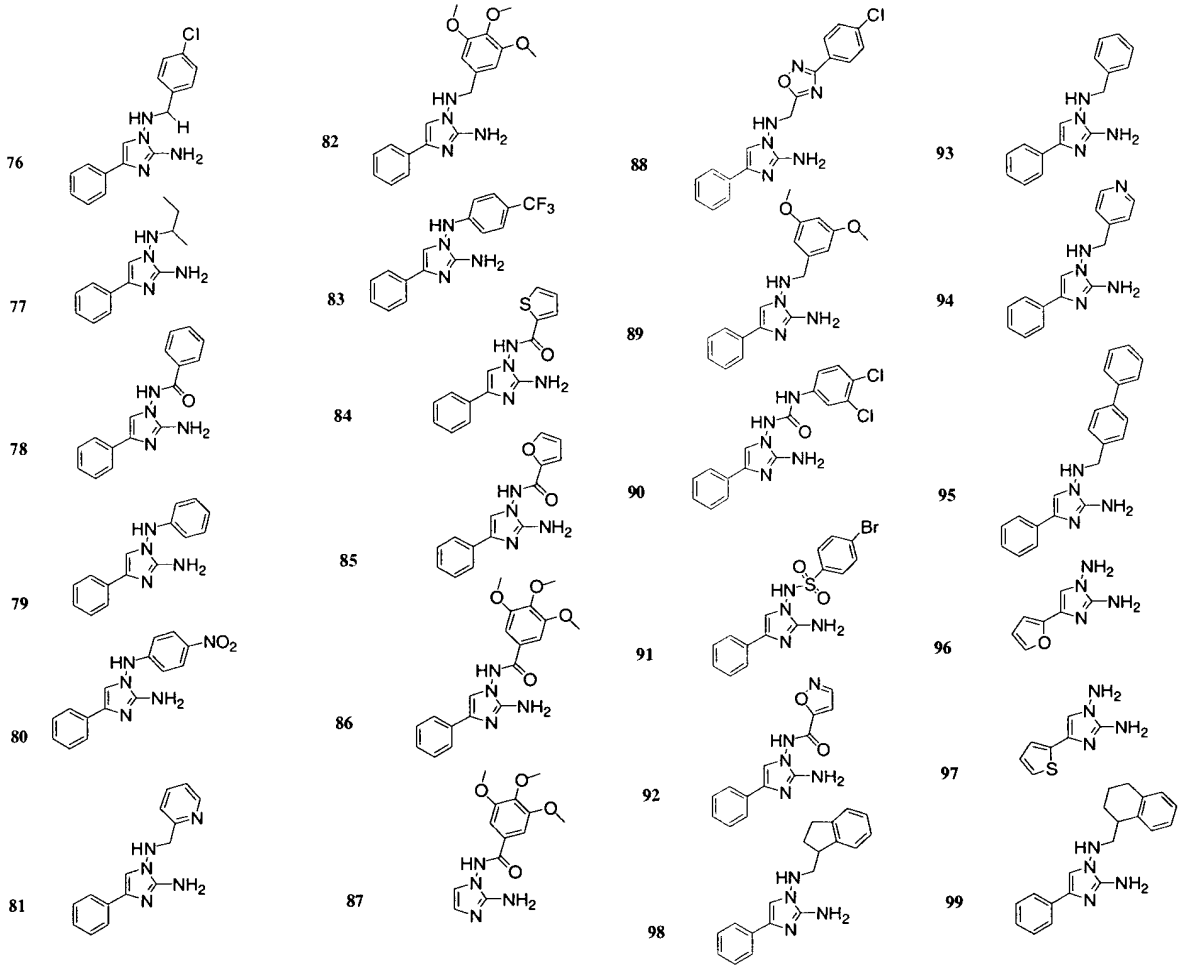


Compound	R^1	R^2	R^3	R^4	R^5
66	4-chlorophenyl	H	H	phenyl	4-chlorobenzyl
67	4-fluorophenyl	H	H	phenyl	[5-(dimethylamino)-1-naphthyl]sulfonyl
68	4-chlorophenyl	H	H	phenyl	4-[5-(dimethylamino)-1-naphthyl]sulfonyl
69	4-chlorophenyl	H	H	phenyl	1,3-benzodioxole-5-carbonyl
70	4-chlorophenyl	H	H	phenyl	2-naphthalenecarbonyl
71	4-chlorophenyl	H	H	phenyl	4-bromo-1-benzenecarbonyl
72	4-fluorophenyl	H	H	phenyl	4-cyano-1-benzenecarbonyl

73	4-fluorophenyl	H	H	phenyl	(1-methyl-1*H*-2-imidazolyl)methyl
74	4-chlorophenyl	H	H	phenyl	4-bromo-1-benzenesulfonyl
75	4-fluorophenyl	H	H	phenyl	4-bromo-1-benzenesulfonyl

Examples 76-99:

The following compounds were synthesized according to the methods described above:



Example 100:

***In vitro* cytotoxicity study**

Human cancer cells gastric NUGC-3, colorectal SW480, lung A549, breast MCF7, uterus MES-SA, and its adriamycin-resistant MES-SA/Dx5 subline were used in *in vitro* cytotoxicity assays. The human cells were seeded at a cell density of 3000 or 4500 cells/100 μl/well in 96-

well flat-bottom plates and incubated for 24 hours at 37°C in a 5% CO_2 incubator. The compounds to be tested were dissolved in dimethyl sulfoxide (DMSO) and further diluted into the culture medium for inhibiting (killing) these human cancer cells *in vitro* to have a final DMSO concentration of 0.3%. Nine compounds of this invention, i.e., Compounds 6, 73, 74, 72, 71, 69, 68, 70, and 75, were prepared in culture media for testing at a range of concentrations from 10, 1, 0.1, 0.01 to 0.001 μM. Each compound solution (200 μl/well) was duplicated in two wells of the cell plates and was treated for 72 hours at 37°C, 5% CO_2 in an incubator. 0.3% DMSO was used as the vehicle control. A colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) and phenazine methosulfate (PMS) was used to determine the potency of the tested compounds. This assay measured cell viability based on the cellular activity in conversion of a tetrazolium salt into a colored soluble formazan product. The optical density (OD) values were measured at 490 nm with a 1420 multilabel counter VICTOR® from Wallac (Turku, Finland). All of the measured values were subtracted with that of the blank control wells without cells before further calculations. The efficacy data are expressed as a percentage normalized to the vehicle controls as calculated in the following formulae. Inhibitory potency (% of vehicle control)=$[(OD490_{compound}-OD490_{blank})/(OD490_{vehicle}-OD490_{blank})]\times 100\%$. The concentration of a test compound that inhibits 50% of the cellular activity (IC_{50}) was determined.

All the tested compounds unexpectedly exhibited a broad spectrum of anticancer activities among all the six used human cancer cell lines.

Example 101:

Evaluation of *in vivo* anticancer activity

The *in vivo* anticancer activities of the compounds were evaluated by the following murine leukemic P388 model. Inbred female DBA/2J mice of 4-5 week-old were purchased from the National Laboratory Animals Breeding and Research Center, Taipei, Taiwan, ROC. Murine leukemic P388 cells were purchased from the Japanese Collection of Research Bioresources, Japan. P388 cells were cultured and propagated in RPMI1640 medium supplemented with 50 μM 2-mercaptoethanol and 10% fetal bovine serum. Mice at the age of 6 weeks were grouped as the treatment, negative control and positive control groups at 7 to 8 mice per group. All mice were intravenously inoculated with the P388 cells at one million per mouse

one day before the treatments initiated. Compound 6 was dissolved in dimethyl sulfoxide (DMSO) and then diluted in 0.5% carboxymethyl cellulose or a Cremophor-based vehicle with the final concentration of DMSO less than 0.5%. Different treatment groups were orally (P.O.) or intravenously (I.V.) given, respectively, with Compound 6 of different doses for a pharmacological dose-response relationship. The mice of the negative control group were treated with the dosing vehicle only. A positive control, doxorubicin 10 mg/kg given by an intravenous injection, was included. The cancer cell-inoculated animals were monitored twice daily. Survival fractions of the mice were recorded. The time on which 50% of the P388-inoculated mice were still surviving is defined as the medium survival time and was used to calculate the percentage (normalized to the medium survival time of the control group) of increased in life span after treatment, which was then served as the index of treatment response.

The results showed that Compound 6 unexpectedly increased the survival rate of the inoculated mice.

OTHER EMBODIMENTS

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.